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Study Plan Summary
J. R. Bridges and J. C. Moser -
Alexandria, LA

ROLE OF TWO PHORETIC MITES IN THE TRANSMISSION OF THE BLUE-STAIN FUNGUS, *CERATOCYSTIS MINOR* (HEDGCOCK 1906)

The southern pine beetle carries several phoretic mites, including at least two that feed on the blue-stain fungus, *Ceratocystis minor*. Because these mycetophagous mites are apt to be contaminated with fungal spores, they could serve as a source of inoculum as they attach to beetles. The objectives of this study are to determine whether the southern pine beetle carries *Ceratocystis minor* spores, whether they are carried by phoretic mites, or whether the spores are transmitted by both.

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RWU 2203

Southern Forest Experiment Station

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Prepared by:

J. Robert Bridges — 2/23/78
Research Entomologist — Date

John C. Moser — 2/24/78
Research Entomologist — Date

Approved by:

Peter L. Lorio, Jr. — 2/27/78
Project Leader — Date
RWU 2203

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ROLE OF TWO PHORETIC MITES IN THE TRANSMISSION OF THE BLUE-STAIN FUNGUS, *CERATOCYSTIS MINOR* (HEDGCOCK 1906)

By

J. Robert Bridges and
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The blue-stain fungus, *Ceratocystis minor* (Hedgcock 1906) Hunt, is usually introduced into a tree at the time of attack by the southern pine beetle (SPB), *Dendroctonus frontalis* Zimmerman 1868 (Dixon and Osgood 1961). After its introduction into the phloem by the beetle, the fungus spreads to the sapwood and contributes to the death of the tree (Bramble and Holst 1935; Nelson and Beal 1929; Nelson 1934). Colonization of the tree by *C. minor* is thought to aid the development of the beetle by reducing the moisture content of the phloem which favors brood development (Nelson 1934). However, direct interaction between developing larvae and blue-stained phloem is detrimental to the beetle (Barras 1970; Franklin 1970), and *C. minor* is apparently inhibited in active beetle galleries, perhaps by the presence of other microorganisms. The fungus does not colonize active beetle galleries but is found after brood development is completed (Oberle 1966; Barras 1970). *C. minor* may be isolated from the body of attacking beetles (Nelson and Beal 1929; Rumbold 1931), but it is not transported, as are some other fungi, in the mycangium (Barras and Perry 1972).

Because *C. minor* does not colonize active beetle galleries, the question arises as to how the beetle becomes inoculated with the fungus. One possible source of inoculum is phoretic mites, especially *Tarsonemus krantzi* Smiley and Moser 1974 and *T. ips* Lindquist 1969 (Moser and Roton 1971; Moser *et al.* 1974). These mites feed on *C. minor* and may be found under the bark in areas where patches of black perithecea of *C. minor* are evident. Mites move from these areas of fungus-colonized bark to cracks in the mid- and outer-bark where they attach to mature adult beetles, usually after the beetles have left their pupal cells (Roton 1978). Mites are apt to be contaminated with *C. minor* spores when they attach to beetles. Therefore, we hypothesize that the phoretic mites are the source of *C. minor* inoculum and that beetles function as transport mechanisms from tree to tree.

OBJECTIVE

The objectives of this study are to determine whether the SPB carry the *C. minor* spores, whether they are carried by phoretic mites, or whether the spores are transmitted by both.

MATERIALS AND METHODS

Incidence of *C. minor* on SPB with or without Mites

To obtain beetles for this part of the study, bolts from a naturally infested loblolly pine tree containing both pupae and callow adults will be brought to the laboratory. The following types of beetles will be assayed to determine the incidence of *C. minor*:

a) Callow adults (without mites)

b) Emergent adults with mites

c) Emergent adults without mites

To obtain mite-free callow adults, the bolts will be dissected and adults removed only from mite-free pupal chambers. Any mature or nearly mature adults in cells will be ignored because at this stage they become attractive to the mites (Roton 1978). Emergent adult SPB will be obtained after they emerge from bolts. About 90% of these beetles contain mites (Roton 1978); therefore, beetles will be inspected for *Tarsonemus* spp., and those with 5 or more mites will be bioassayed for *C. minor*. Emergent beetles that are found by inspection to be free of mites will also be bioassayed. Beetles with or without mites will be macerated in 0.2 ml of 0.85% NaCl, and the macerate will be spread over the surface of a malt agar plate. Fungi that grow from the beetles will be examined to determine the incidence of *C. minor*.

Incidence of *C. minor* on Mites

Tarsonemus krantzi and *T. ips* will be obtained from areas of *C. minor*-colonized bark tissue and from mature SPB after they emerge from naturally infested loblolly pine bolts in the laboratory. Four types of mites will be assayed:

T. ips
ex. mature SPB
ex. *C. minor* colonies

T. krantzi
ex. mature SPB
ex. *C. minor* colonies

The mites will be crushed and plated on malt agar plates. Fungi growing on these plates will be examined to determine the incidence of *C. minor*.

Mites will also be examined microscopically for the presence of *C. minor* spores in or on the mite. Scanning electron microscopy (SEM) will be used to aid in determining the location and distribution of spores on the mites. For SEM, mites will be mounted with silver conductive paint on an aluminum stub. After drying in a desiccator, the mites will be coated with palladium-gold and examined by SEM.

Incidence of *C. minor* in Larval and Pupal Chambers

To determine whether beetles obtain *C. minor* inoculum from bark surrounding the pupal chambers, miteless chambers will be scraped and cultured on malt agar. Trees with thin bark (<2 mm) as well as ones with thick bark (>4 mm) will be sampled. SPB pupate in the outer bark except in trees with thin bark where they pupate directly in larval cells next to the xylem (Roton 1978). The latter may be a potential source of *C. minor* spores for emerging brood adults.

Statistical Analysis of the Data

Data for the incidence of *C. minor* on SPB will be statistically analyzed using a chi square test of independence as illustrated in the following 2 by 3 contingency table:

	Group I	Group II	Group III	Subtotals
with *C. minor*	a_1	a_2	a_3	A
without *C. minor*	b_1	b_2	b_3	B
Subtotals *	T_1	T_2	T_3	G

Group I = Callow adults
Group II = Emergent beetles without mites
Group III = Emergent beetles with mites

*100 or more beetles (or mites) of each type will be assayed.

Values for chi square (X^2) will be calculated from:

$$\chi^2 = \frac{1}{(A)(B)} \sum_{i=1}^{3} \left(\frac{[a_i B - b_i A]^2}{T_i} \right)$$

The chi square test of independence will also be used to analyze data for the incidence of *C. minor* on mites and in pupal chambers from thin and thick bark trees. For mites, Group I = *T. krantzi* from beetles, Group II = *T. ips* from beetles, Group III = *T. krantzi* from *C. minor* colonies, and Group IV = *T. ips* from *C. minor* colonies. For pupal chambers, Group I = pupal chambers from thin bark trees and Group II = pupal chambers from thick bark trees.

DATES OF ESTABLISHMENT AND COMPLETION

The study will begin in January 1978 and will be completed in 18 months.

PRESENTATION OF RESULTS

A final report and manuscript(s) will be prepared upon completion of the study.

PERSONNEL ASSIGNMENT

J. Robert Bridges	Research Entomologist
John C. Moser	" "
Thelma Perry	Laboratory Technician
John W. Monahan	" "
Lary M. Roton	" "

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